

**SECTION 6A – BSL4/ABSL4 LABORATORIES ONLY: TO BE COMPLETED BY ALL ENTITIES
FOR EACH PRINCIPAL INVESTIGATOR**

NOTE: All entities must also complete Section 5A, Questions 1 and 2, above (CDC Form 0.1319/APHIS Form 2040)

47. All entities must answer the following questions for each BSL-4 laboratory:

a. Activities conducted under BSL-4 containment (check all that apply):

Research Diagnostic Large scale production Small animal Large animal
Recombinant DNA Other (give description): _____

b. How many separate BSL-4 laboratories are you registering for select agent work?

1 laboratory 2 laboratories 3 or more laboratories

c. Are these laboratories currently registered with the CDC Select Agent Program? Yes No

d. Are these laboratories currently registered with the APHIS? Yes No

e. Are these BSL-4 laboratories currently operational (presently conducting BSL-4 work)? Yes No

f. What type of BSL-4 laboratory (ies) are you registering?

Protective suit laboratory Stand alone Class III cabinet laboratory
Protective suit laboratory with associated Class III cabinet

48. Include a floor plan for each BSL-4 laboratory, Class III cabinet laboratory, or ABSL-4 laboratory where select agents are to be used or stored.

Floor plan(s) must include:

a. Sink locations	Yes	No
b. Eyewash locations	Yes	No
c. Laboratory furniture locations (including bench work)	Yes	No
d. Biosafety cabinet (BSC) locations	Yes	No
e. Fume hood locations	Yes	No
f. HVAC supply and exhaust locations	Yes	No
g. Freezer/refrigerator locations (include LN2 storage)	Yes	No
h. Other large equipment locations (e.g., incubators, centrifuges)	Yes	No

49. Provide information on the biosafety cabinets in use (attach additional sheets if needed):

a. Class of cabinet: II, Type A1 II, Type A2 (formerly II, B3) II, B1 II, B2 Class III
b. Biosafety cabinet connection to the HVAC system: Hard ducted Thimble Re-circulating
c. Define certification period: Annual Biannual Other (explain): _____

50. Provide a description of the BSL-4 HVAC system (*check all that are appropriate*):

a. Single-pass
b. Dedicated exhaust
c. Constant air volume Variable air volume
d. Redundant exhaust fans
e. Emergency power back-up

51. Provide general facility and safety information for the BSL-4 laboratory facility (ies) you are registering by answering the questions in this section. Use separate sheets if necessary.

- a. BSL-4 laboratory design and operational procedures are documented and re-verified annually: Yes No
- b. A specific BSL-4 facility operations manual has been prepared: Yes No
- c. All standard BSL-4 microbiological practices are followed: Yes No
- d. There is a mandatory daily inspection of the containment parameters for the BSL-4 laboratory area(s) and critical life support systems: Yes No
- e. Walls, floors, and ceilings of the BSL-4 laboratory rooms are sealed. All penetrations into the laboratory are sealed: Yes No
- f. The HVAC system is dedicated and is not re-circulated: Yes No
- g. There is a visual and auditory alarm system provided to alert facility workers to system malfunctions and/or failures of containment parameters: Yes No
- h. Entry to the laboratory is through a double set of lockable, self-closing doors: Yes No
- i. Each protective suit or cabinet laboratory room has a hands-free sink: Yes No
- j. There is a double door autoclave for decontamination of materials from the suit lab and/or the Class III cabinet and cabinet room: Yes No
- k. A visual pressure differential monitoring system is provided at the clean change room for laboratory personnel to verify directional air before entry into the BSL-4 laboratory: Yes No
- l. Differential pressures/directional airflow between adjacent areas is monitored and alarmed (visually and audibly) to indicate system failure: Yes No
- m. Double HEPA filtration of all suit area, decontamination shower, decontamination airlock and Class III cabinet exhaust air is in place: Yes No
- n. Single HEPA filtration of all suit area, decontamination shower, decontamination airlock and Class III cabinet supply air is in place: Yes No
- o. Describe method utilized for decontamination of BSL-4 area(s):

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- p. Inactivation of organisms and materials removed from BSL-4 containment is accomplished by what method?
Irradiation Chemical disinfection Autoclaving Other
Describe: _____

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- q. Inactivation of materials removed from BSL-4 containment is verified: Yes No
Describe: _____

Facilities registering a laboratory containing a Class III cabinet, must answer question 52. Facilities wishing to register protective suit laboratories and suit laboratories with associated Class III cabinets must also answer question 53.

52. Entities registering a **stand alone Class III Cabinet laboratory registration** must verify the following items:

- a. Entry to the laboratory housing the Class III cabinet is through a double set of lockable, self-closing doors: Yes No
- b. Inner and outer change rooms are separated by a shower for personnel entering and leaving the cabinet room: Yes No
- c. There is a double-door (pass-through) autoclave, dunk tank, fumigation chamber, or ventilated anteroom for passing materials, supplies, or equipment into or out of the cabinet room: Yes No

- d. Walls, floors, and ceilings of the cabinet room(s) are sealed and all penetrations into the cabinet room(s) are sealed: Yes No
- e. Floors are seamless and coved: Yes No
- f. All drains in the cabinet room(s), inner change room(s), and autoclave chambers connect directly to an appropriate liquid waste decontamination system: Yes No
- g. Sewer vents and other service lines contain HEPA filters: Yes No
- h. Bench tops are seamless or sealed surfaces that are impervious to water and resistant to moderate heat and organic solvents, acids, alkalis, and other decontaminant chemicals: Yes No
- i. Laboratory furniture is capable of supporting anticipated loads and uses and is covered with a non-fabric material that can be easily decontaminated: Yes No
- j. A hands-free sink is located in the cabinet room(s) near the door and in the inner and outer change rooms: Yes No
- k. If a central vacuum system is present, it serves only the cabinet room(s) and is HEPA filter protected, and liquid and gas services to the cabinet room are protected by backflow prevention devices: Yes No
- l. Any windows are break resistant and sealed: Yes No
- m. Double-door autoclaves are provided for decontamination of materials removed from the Class III cabinet and the cabinet room. These autoclaves are interlocked so that the outside door can only be opened after the sterilization cycle is complete: Yes No
- n. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s): Yes No
- o. All HEPA filters are tested and certified annually: Yes No
- p. An HVAC monitoring system is provided to avoid pressurization of the laboratory and is alarmed to warn laboratorians of exhaust system failure: Yes No
- q. There is HEPA filtration of all supply and exhaust air from the cabinet room(s), inner change room(s), and anteroom(s): Yes No
- r. The Class III cabinet is directly connected to the exhaust system with HEPA filtration on the supply and double HEPA filtration on the exhaust: Yes No
- s. Appropriate communication systems are provided between the laboratory and external personnel (intercom, phone, fax, and computer): Yes No

53. Entities registering a **protective suit laboratory or a protective suit laboratory with associated Class III cabinet registration** must verify the following items (**suit laboratories with associated Class III cabinets must also answer question 52:**):

- a. Entry into the area(s) where work is performed with BSL-4 agents [suit room(s)] is through a series of changing and decontamination areas separated by airtight doors: Yes No
- b. Inner and outer change rooms are separated by a personal shower: Yes No
- c. A chemical shower is provided for decontaminating the outer surface of the protective suit: Yes No
- d. A breathing air system is provided with redundant compressors, backup storage tanks, HEPA filtration protection, and alarm monitoring in the event of failure: Yes No
- e. All penetrations into containment shell (walls, floors, and ceilings) of the suit area(s), chemical shower(s), and airlock(s) are sealed: Yes No
- f. Daily inspections of the containment parameters and life support systems are performed, completed and documented before laboratory work begins: Yes No
- g. A double-door, interlocked autoclave is provided for decontaminating waste materials removed from the suit area(s): Yes No
- h. A dunk tank, fumigation chamber, or ventilated airlock to pass materials, supplies, or equipment into or out of the suit area(s): Yes No

- i. Bench tops are seamless surfaces that are impervious to water and resistant to moderate heat and organic solvents, acids, alkalis, and other decontaminant chemicals: Yes No
- j. Laboratory furniture is capable of supporting anticipated loads and uses and is covered with a non-fabric material that can be easily decontaminated: Yes No
- k. A hands-free sink is located in the suit area(s): Yes No
- l. If a central vacuum system is present, it serves only the suit area(s) and is protected by HEPA filtration: Yes No
- m. Liquid and gas services to the suit area(s) are protected by backflow devices: Yes No
- n. Inner and outer doors to chemical showers and airlocks are interlocked to prevent both doors from being opened at the same time: Yes No
- o. Any windows are break resistant and sealed: Yes No
- p. All drains in the suit area(s), chemical shower(s), and autoclave chambers connect directly to an appropriate liquid waste decontamination system: Yes No
- q. An HVAC monitoring system is provided to avoid pressurization of the laboratory and is alarmed to warn laboratorians in the event of exhaust system failure: Yes No
- r. Redundant exhaust fans are installed: Yes No
- s. All HEPA filters are tested and certified annually: Yes No
- t. HVAC supply to the suit area(s), chemical shower(s), and airlock(s) is HEPA filtered: Yes No
- u. HVAC exhaust from the suit area(s), chemical shower(s), and airlock(s) is double HEPA filtered with the HEPA filters in series: Yes No
- v. Appropriate communication systems are provided between the laboratory and external personnel (intercom, phone, fax, and computer): Yes No
- w. Emergency lighting and emergency communications systems are provided for the BSL-4 areas: Yes No

54. Entities registering an **ABSL-4 laboratory** must provide the following information. Entities registering a **stand alone Class III cabinet** for housing animals infected with biosafety level 4 agents, or other ABSL-4 use must complete **question 52** above. Entities registering a **protective suit laboratory** housing animals infected with Biosafety level 4 agents must complete **question 53**:

- a. List animal models in use for ABSL-4 experiments: _____
- b. ABSL-4 Laboratory Room(s) designations: _____
- c. Specific procedures have been developed for handling animals under ABSL-4 conditions in the Class III cabinet or Protective suit laboratory(ies) being registered: Yes No
- d. All appropriate special policies and procedures are approved by the Institutional Animal Care and Use Committee: Yes No
- e. Are aerosol experiments conducted in this ABSL-4 laboratory (ies): Yes No
- f. Describe how are animals housed under ABSL-4 conditions: _____
- g. Cage washing is with a mechanical cage washer: Yes No
- h. Cage washing area is shown on the floor plans: Yes No
- i. Animal waste is sterilized (carcasses, sewage, bedding, etc.) before disposal Yes No
Describe treatment method: _____
- j. Method of disposal of treated carcasses? Incineration Rendering Chemical decomposition
Other (describe): _____
- k. If floor drains are provided, the traps are always filled with an appropriate disinfectant: Yes No

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| l. Appropriate personal protective equipment is used: | Yes | No |
| m. Personnel assigned to work with infected animals work in pairs: | Yes | No |

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|--|-----|----|--------------------------|
| 55. Vacuum lines contain HEPA filters: | Yes | No | No vacuum lines are used |
| 56. A medical surveillance system is in place for laboratory personnel using select agents: | Yes | No | |
| 57. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director: | Yes | No | |
| 58. A sharps policy is in place for this laboratory (or laboratories): | Yes | No | |
| 59. A site-specific emergency operations plan is available for this laboratory: | Yes | No | |
| 60. An Institutional Biosafety Committee (IBC) reviews and approves protocols prior to work with select agents at this entity? | Yes | No | |
| a. If yes, has IBC approved the work proposed in this application: | Yes | No | |
| b. The entity has been inspected by USDA, FDA, CLIA, DoE, DoD or others: | Yes | No | |
| c. If yes, then give agency and date of last inspection(s): _____ | | | |
| 61. Briefly state (no more than a paragraph) the objectives of the work with the select agent(s), including a description of the methodologies or laboratory procedures that will be used. State if any host-vector systems will be used. Specify whether work will involve live agents and recombinant DNA: | | | |
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SECTION 6B – BSL4/ABSL4 LABORATORIES ONLY: TO BE COMPLETED BY ALL ENTITIES (TRAINING AND SECURITY)

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| 62. Training: | | | |
| a. Site-specific security training is provided to individuals with access to areas where BSL-4 select agents are handled or stored: | Yes | No | |
| b. Site-specific safety training is provided to individuals with access to areas where BSL-4 select agents are handled or stored: | Yes | No | |
| c. A biosafety manual has been prepared that indicates special hazards associated with the BSL-4 agents in use and laboratory personnel are required to read and follow these practices and procedures: | Yes | No | |
| d. Training is provided to laboratory personnel prior to beginning work with BSL-4 select agents: | Yes | No | |
| e. Training is provided: Annually Biannually Other (specify frequency): _____ | | | |
| f. Written records of individuals trained are kept: | Yes | No | |
| g. Personnel are required to demonstrate proficiency in laboratory procedures prior to working with BSL-4 select agents: | Yes | No | |
| h. Please provide a brief description of the individual training program for BSL-4 laboratory personnel (attach additional sheets if necessary): | | | |
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63. Security: Provide a brief explanation of the system in place to detect loss or theft of select agent(s):

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| a. All viable BSL-4 agents are stored within the BSL-4 containment area: | Yes | No |
| b. Storage areas within BSL-4 containment are under surveillance: | Yes | No |
| c. Individual responsible for inventory of select agent(s): _____ | | |
| d. How often is the inventory record reconciled? _____ | | |
| e. How is access to the inventory log limited? _____ | | |
| f. Inventory tracking includes the following information (list): | | |

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| 64. There is a site-specific security plan for each of the BSL-4 laboratories listed above: | Yes | No |
| a. Only persons whose presence in the BSL-4 laboratory facility or individual laboratory rooms is required for program or support purposes are authorized to enter: | Yes | No |
| b. Access to the laboratory is controlled by secure, locked doors: | Yes | No |
| c. A signature log book indicating date and time of entry and exit of all personnel to and from the BSL-4 containment area is maintained: | Yes | No |
| d. Indicate means of limiting access to buildings with BSL-4 laboratories using select agents: | | |
| Guard station at the entity entrance | | |
| Card access system or locks | | |
| Security alarm system in the laboratory building | | |
| Other (describe): _____ | | |
| e. Indicate means of limiting access to select agents once inside the building: | | |
| Door to laboratory is locked | | |
| Guard station at the building entrance | | |
| Card access system or locks | | |
| Security alarm system in the laboratory | | |
| Other (describe): _____ | | |
| f. Means to limit access to select agents once inside the laboratory: | | |
| Locked incubators, refrigerators, freezers, etc. | | |
| Security alarm system that directly monitors the laboratory | | |
| Other (describe): _____ | | |
| g. Means to limit access to select agents in storage: | | |
| Storage area door locked | | |
| Lock boxes | | |
| Security alarm system that directly monitors the laboratory | | |
| Other (describe): _____ | | |
| h. Means to monitor unauthorized entry into the BSL-4 laboratory where select agents are used or stored: | | |
| Electronic logs of card access system entries are reviewed for unusual activity | | |
| Manual sign in and out logs are kept and monitored | | |
| Camera surveillance (e.g., CCTV) | | |
| Other (describe): _____ | | |
| i. The laboratory is secured when no one is present during regular working hours: | Yes | No |
| j. The laboratory is secured when no one is present after regular working hours: | Yes | No |
| k. Total number of personnel with access to BSL-4 area during operations: _____ | | |
| l. Individuals not directly involved in research activities have access to select agents: | Yes | No |
| If yes, please explain: _____ | | |

- m. Non-laboratory personnel (visitors, including janitorial and facility maintenance personnel) have access to the laboratory with select agents: Yes No
 If yes, are they allowed into the laboratory unescorted? Yes No
 If yes, please explain: _____
- n. Describe how the entity limits access to the laboratories where select agents are being manipulated and stored to only authorized and qualified persons: _____

**SECTION 6C – BSL4/ABSL4 LABORATORIES ONLY: TO BE COMPLETED BY ENTITIES
WORKING WITH INFECTIOUS AGENTS**

65. Provide an estimate of the maximum quantities (e.g., number of petri dishes or flasks) and concentration of organisms grown at any given time: _____
- a. All cultures, stock and other regulated wastes are decontaminated before disposal by an approved sterilization method: Yes No
 If yes, describe method: _____

**SECTION 6D – BSL4/ABSL4 LABORATORIES ONLY: TO BE COMPLETED BY ENTITIES
WORKING WITH RECOMBINANT DNA**

66. This laboratory meets NIH guidelines for research involving recombinant DNA molecules: Yes No
67. Will you possess, use or transfer the following:
- a. Select agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that are capable of infection and/or replication. Yes No
- b. Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the toxins listed in paragraph (d) of this section if the nucleic acids are in a vector or host chromosome and/or are expressed *in vivo* or *in vitro*. Yes No
- c. Select agent viruses, bacteria, fungi, and toxins that have been genetically modified. Yes No
68. Do you intend to conduct the following experiments:
- a. Experiments utilizing recombinant DNA that involve the deliberate transfer of a drug resistance trait to select agents that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. Yes No
- b. Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD₅₀ < 100 ng/kg body weight. Yes No
69. Provide a brief description of the recombinant constructs and any associated expression control elements, including what the recombinant DNA encodes for, if known: _____
70. Give an estimate of range of length of recombinant DNA to be used: _____

**SECTION 6E – BSL4/ABSL4 LABORATORIES ONLY: TO BE COMPLETED BY ENTITIES
WORKING WITH SMALL ANIMALS**

71. List species of small animals that will be used: _____
72. Describe route of infection: _____
73. Animal waste is treated prior to disposal (e.g., carcasses, sewage, bedding, etc.): Yes No
 If yes, describe method: _____
74. The entity requires that an Institutional Animal Care and Use Committee (IACUC) review and approve protocols prior to work with animals at this entity: Yes No
- a. If yes, the proposed work with select agents in small animals has been approved by the IACUC: Yes No
- b. The laboratory space is accredited by AAALAC: Yes No

c. If yes, give inspection date: _____

**SECTION 6F – BSL4/ABSL4 LABORATORIES ONLY: TO BE COMPLETED BY ENTITIES
WORKING WITH LARGE ANIMALS**

75. List species of large animals that will be used: _____
- a. Describe route of infection: _____
- b. Carcass of animals are disposed of to avoid their use as food for human beings or animals: Yes No
- c. Animal waste is treated prior to disposal (e.g., carcasses, sewage, bedding, etc.): Yes No
- If yes, give method: _____
76. Carcass of animals are disposed on site: Yes No
- a. The entity requires that an Institutional Animal Care and Use Committee (IACUC) review and approve protocols prior to work with animals at this entity: Yes No
- If yes, the proposed work with select agents in small animals has been approved by the IACUC: Yes No
77. The laboratory space is accredited by AAALAC: Yes No

Attachments

Attachment 1. 42 CFR Part 73. Select Biological Agents and Toxins; Final Rule. Federal Register, December 13, 2002.

Attachment 2. 9 CFR Part 121 - Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins. Federal Register, December 13, 2002.

Attachment 3. 7 CFR 331 – Possession of Select Agents. Federal Register, December 13, 2002.

Attachment 4. APHIS application for permit to import or transport controlled material or organisms or vectors (VS form 16-3). (see pdf attachment)

Attachment 5. Additional Information for cell cultures and their products (VS form 16-7). (see pdf attachment)

Attachment 6. Guidance document for report of transfer of select agents and toxins and EA-101

The purpose of the CDC EA-101 form is to provide a method for the documentation of the transfer of a select agent. An EA-101 form must be completed for each transfer of a select agent. A copy of each EA-101 must be kept by the responsible official (RO) for three years.

Prior to transferring a select agent

Before a select agent is transferred, both the sender (transferor) and recipient (requestor) facilities must be registered with the CDC or APHIS. The agency that the Responsible Official (RO) should contact is determined by the type of select agent or toxin involved in the transfer. For HHS agents, the RO should contact CDC by facsimile (404-498-2265). For USDA agents, the RO should contact APHIS (for animal agents and toxins, telephone: 301-734-3277; facsimile: 301-734-3652). For HHS/USDA overlap agents, the RO should contact either APHIS or CDC. For plant agents and toxins the RO should contact APHIS (telephone: 301-734-5519; facsimile: 301-734-8700). A listing of HHS select agents and toxins is available at <http://www.cdc.gov/od/sap>. A listing of USDA animal agents and toxins is available at <http://www.aphis.usda.gov/vs/ncie/bta.html>. The list of plant agents and toxins is available at <http://www.aphis.usda.gov/ppq/permits>.

The recipient fills out blocks 1 and 2 of the EA-101 form and submits it to the sender. The sender's responsible official (RO) must FAX the form to CDC (FAX: 404-498-2265) or APHIS (FAX: 301-734-3652) to verify that the requesting entity: (1) retains a valid, current registration for the select agent being requested; (2) the person requesting the select agent is an employee of the requesting entity, and has been given Department of Justice clearance as an authorized individual to receive the select agent material to be transferred; and, (3) that the proposed use of the agent by the recipient is correctly indicated on CDC Form EA-101. CDC or APHIS will FAX back the form with a confirmation if the transfer information is approved. If the sender has a suspicion that the agent may not be used for the requested purpose, or there are any other concerns, then the sender should consult with the CDC.

Transfer:

(a) Shipment of the select agent to the recipient

The sender should ship the material to the receiver only after the sender has received a verification number from CDC or APHIS regarding the information in blocks 1 and 2 of the EA-101. The sender fills out Section 4, including the date the agent was shipped. Select agents must be packaged, labeled, and shipped in accordance with all federal regulations (e.g., 42 CFR 72, and 49 CFR 100-180) and international (IATA) regulations. It is highly recommended that the sender utilize a method for tracking the movement of the select agents being shipped.

(b) Transmittal of the EA-101 form to the CDC or APHIS

The RO from the recipient's entity must fill out Section 4 of the EA-101 form with the date received and FAX the form back to both the Sender's RO and the CDC or APHIS. The recipient is required to provide a completed paper copy or facsimile transmission of the EA-101 form within 2 business days to the Sender RO and the CDC or APHIS.

Destruction or depletion of a select agent

When a select agent from a transfer is depleted or destroyed, the RO of the entity must complete the appropriate information in Block 4 of the Form. A copy or FAX of the EA-101 form must be sent to the CDC or APHIS.

Recipient RO	Sender RO
1. Completes agent description (Block 1)	
2. Completes recipient information (Block 2)	
3. Faxes form EA-101 and registration certificate to sender	
	4. Completes sender information (Block 3)
	5. Faxes form EA-101 to CDC or APHIS for verification number
	6. After receipt of approval by CDC or APHIS, sender completes shipping information (Block 4), except for date received
	7. Oversees packaging and shipment of agent to recipient. Sends shipment.
8. Receives agent	
9. Recipient RO completes Block 4 (i.e., date select agent material received and confirms that what was listed on packing inventory has been received) and provides paper copy or faxes form EA-101 to both CDC or APHIS and the sender within 2 business days of receipt.	
10. Retains paper record for 3 yr, or retains record 3 yr after agent consumed or destroyed, whichever is longer	10. Retains paper record for 3 yr, or retains record 3 yr after agent consumed or destroyed, whichever is longer